CLAIMS

What is claimed is:

1. An apparatus for determining a threshold cycle number in a nucleic acid amplification reaction, the apparatus comprising:

a) a detection mechanism for measuring, at a plurality of different times during the amplification reaction, at least one signal whose intensity is related to the quantity of a nucleic acid sequence being amplified in the reaction; and

b) a controller in communication with the detection mechanism, wherein the controller is programmed to perform the steps of:

i) deriving a growth curve from the measurements of the signal;

ii) calculating a derivative of the growth curve;

iii) identifying a characteristic of the derivative; and

iv) determining a cycle number associated with the characteristic of the derivative.

2. The apparatus of claim 1, wherein the controller is programmed to calculate a second derivative of the growth curve, and wherein the characteristic comprises a positive peak of the second derivative.

3. The apparatus of claim 1, wherein the controller is programmed to calculate a second derivative of the growth curve, and wherein the characteristic comprises a negative peak of the second derivative.

4. The apparatus of claim 1, wherein the controller is programmed to calculate a second derivative of the growth curve, and wherein the characteristic comprises a zero crossing of the second derivative.

5. The apparatus of claim 1, wherein the controller is programmed to calculate a first derivative of the growth curve, and wherein the characteristic comprises a positive peak of the first derivative.

6. The apparatus of claim 1, wherein the controller is programmed to calculate second derivative values of the growth curve at a number of different cycles in the reaction to yield a plurality of second derivative data points, the characteristic comprises a positive peak of the second derivative, and the controller is further programmed to determine the cycle number associated with the positive peak by:

i) fitting a second order curve to the second derivative data points; and

ii) calculating the cycle number as the location, in cycles, of a peak of the second order curve.

7. An apparatus for determining a threshold time value in a nucleic acid amplification reaction, the apparatus comprising:

a) a detection mechanism for measuring, at a plurality of different times during the amplification reaction, at least one signal whose intensity is related to the quantity of a nucleic acid sequence being amplified in the reaction; and

b) a controller in communication with the detection mechanism, wherein the controller is programmed to perform the steps of:

i) deriving a growth curve from the measurements of the signal;

ii) calculating a derivative of the growth curve;

iii) identifying a characteristic of the derivative; and

iv) determining a time value associated with the characteristic of the derivative.

8. The apparatus of claim 7, wherein the controller is programmed to calculate a second derivative of the growth curve, and wherein the characteristic comprises a positive peak of the second derivative.

9. The apparatus of claim 7, wherein the controller is programmed to calculate a second derivative of the

growth curve, and wherein the characteristic comprises a negative peak of the second derivative.

10. The apparatus of claim 7, wherein the controller is programmed to calculate a second derivative of the growth curve, and wherein the characteristic comprises a zero crossing of the second derivative.

11. The apparatus of claim 7, wherein the controller is programmed to calculate a first derivative of the growth curve, and wherein the characteristic comprises a positive peak of the first derivative.

12. The apparatus of claim 7, wherein the controller is programmed to calculate second derivative values of the growth curve at a plurality of different measurement times in the reaction to yield a plurality of second derivative data points, the characteristic comprises a positive peak of the second derivative, and the controller is further programmed to determine the time value associated with the positive peak by:

i) fitting a second order curve to the second derivative data points; and

ii) calculating the time value as the location of a peak of the second order curve.

13. An apparatus for determining an unknown starting quantity of a target nucleic acid sequence in a test sample, the apparatus comprising:

means for amplifying the unknown starting quantity of the target nucleic acid sequence in the test sample and for amplifying a plurality of known starting quantities of a calibration nucleic acid sequence in respective calibration samples;

at least one detection mechanism for measuring, at a plurality of different times during amplification of the nucleic acid sequences, signals indicative of the quantities of the nucleic acid sequences being amplified in the test and calibration samples; and

at least one controller in communication with the detection mechanism, wherein the controller is programmed to perform the steps of:

a) determining a respective threshold value for each of the known starting quantities of the calibration nucleic acid sequence in the calibration samples and for the target nucleic acid sequence in the test sample, wherein each threshold value is determined for a nucleic acid sequence in a respective sample by:

i) deriving a growth curve for the nucleic acid sequence from the measured signals;

ii) calculating a derivative of the growth curve;

iii) identifying a characteristic of the derivative; and

iv) determining the threshold value associated with the characteristic of the derivative;

b) deriving a calibration curve from the threshold values determined for the known starting quantities of the nucleic acid sequence in the calibration samples; and

c) determining the starting quantity of the target nucleic acid sequence in the test sample using the calibration curve and the threshold value determined for the target sequence.

14. The apparatus of claim 13, wherein each of the threshold values comprises a cycle number.

15. The apparatus of claim 13, wherein each of the threshold values comprises an elapsed time of amplification.

16. The apparatus of claim 13, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a positive peak of the second derivative.

17. The apparatus of claim 13, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a negative peak of the second derivative.

18. The apparatus of claim 13, wherein the step of calculating a derivative of the growth curve comprises calculating a second derivative of the growth curve, and wherein the characteristic comprises a zero crossing of the second derivative.

19. The apparatus of claim 13, wherein the step of calculating a derivative of the growth curve comprises calculating a first derivative of the growth curve, and wherein the characteristic comprises a positive peak of the first derivative.

20. The apparatus of claim 13, wherein the step of calculating a derivative of the growth curve comprises calculating second derivative values of the growth curve at a number of different measurement points to yield a plurality of second derivative data points, the characteristic comprises a positive peak of the second derivative, and the step of determining the threshold value associated with the positive peak comprises:

i) fitting a second order curve to the second derivative data points; and

ii) calculating the threshold value as the location of a peak of the second order curve.

21. The apparatus of claim 1, wherein the controller is further programmed to terminate the amplification reaction when the characteristic is identified.

22. The apparatus of claim 7, wherein the controller is further programmed to terminate the amplification reaction when the characteristic is identified.

23. An apparatus for determining a threshold value in a nucleic acid amplification reaction, the apparatus comprising:

a) at least one detection mechanism for measuring, at a plurality of different times during the amplification reaction, at least one signal whose intensity is related to the quantity of a nucleic acid sequence being amplified in the reaction; and

b) a controller in communication with the detection mechanism, wherein the controller is programmed to perform the steps of:

i) storing signal values defining a growth curve for the nucleic acid sequence, wherein the growth curve expresses signal intensity as a

function of cycle number or as a function of time in the reaction;

ii) determining a derivative of the growth curve, wherein the derivative is determined with respect to cycle number or time; and

iii) calculating a cycle number or time value associated with a characteristic of the derivative.

24. The apparatus of claim 23, wherein the controller is further programmed to identify the characteristic of the derivative as the amplification reaction is occurring and to terminate the amplification reaction when the characteristic is identified.

25. The apparatus of claim 23, wherein the controller is programmed to determine the second derivative of the growth curve, and wherein the cycle number or time value is calculated as the location, in cycles or in time of amplification, of a maximum of the second derivative.

26. The apparatus of claim 23, wherein the controller is programmed to determine the second derivative of the growth curve, and wherein the cycle number or time value is calculated as the location, in cycles or in time of amplification, of a minimum of the second derivative.

27. The apparatus of claim 23, wherein the controller is programmed to determine the second derivative of the growth curve, and wherein the cycle number or time value is calculated as the location, in cycles or in time of amplification, of a zero-crossing of the second derivative.

28. The apparatus of claim 23, wherein the controller is programmed to determine the first derivative of the growth curve, and wherein the cycle number or time value is calculated as the location, in cycles or in time of amplification, of a maximum of the first derivative.

29. The apparatus of claim 23, wherein the characteristic of the derivative comprises a maximum of the second derivative, and wherein the controller is programmed to perform steps (ii) and (iii) by:

calculating second derivative values of the growth curve, with respect to cycle number or time, at a number of different measurement points to yield a plurality of second derivative data points;

fitting a second curve to at least three of the second derivative data points; and

calculating the cycle number or time value as the location, in cycles or in time of amplification, of a positive peak of the second curve.

30. The apparatus of claim 29, wherein the cycle number or time value at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three second derivative data points.

31. The apparatus of claim 23, wherein the characteristic of the derivative comprises a minimum of the second derivative, and wherein the controller is programmed to perform steps (ii) and (iii) by:

calculating second derivative values of the growth curve, with respect to cycle number or time, at a number of different measurement points to yield a plurality of second derivative data points;

fitting a second curve to at least three of the second derivative data points; and

calculating the cycle number or time value as the location, in cycles or in time of amplification, of a negative peak of the second curve.

32. The apparatus of claim 31, wherein the cycle number or time value at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three second derivative data points.

33. The apparatus of claim 23, wherein the characteristic of the derivative comprises a maximum of the first derivative, and wherein the controller is programmed to perform steps (ii) and (iii) by:

calculating first derivative values of the growth curve, with respect to cycle number or time, at a number of different measurement points to yield a plurality of first derivative data points;

fitting a second curve to at least three of the first derivative data points; and

calculating the cycle number or time value as the location, in cycles or in time of amplification, of a peak of the second curve.

34. The apparatus of claim 33, wherein the cycle number or time value at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three first derivative data points.

35. The apparatus of claim 23, wherein the characteristic of the derivative comprises a zero-crossing of the second derivative, and wherein the controller is programmed to perform steps (ii) and (iii) by:

calculating second derivative values of the growth curve at a number of different measurement points to yield a plurality of second derivative data points; and

calculating the cycle number or time value at the zero-crossing by interpolation between at least two of the second derivative data points.

36. An apparatus for determining an unknown starting quantity of a target nucleic acid sequence in a test sample, the apparatus comprising:

means for amplifying the unknown starting quantity of the target nucleic acid sequence in the test sample and for amplifying a plurality of known starting quantities of a calibration nucleic acid sequence in respective calibration samples;

at least one detection mechanism for measuring, at a plurality of different times during amplification of the nucleic acid sequences, signals indicative of the quantities of the nucleic acid sequences being amplified in the test and calibration samples; and

at least one controller in communication with the detection mechanism, wherein the controller is programmed to perform the steps of:

a) determining a respective threshold value for each of the known starting quantities of the calibration nucleic acid sequence in the calibration samples and for the target nucleic acid sequence in the test sample, wherein each threshold value is determined for a nucleic acid sequence in a respective sample by:

1) storing signal values defining a growth curve for the nucleic acid sequence, wherein the growth curve expresses signal intensity as a function of cycle number or as a function of time in the reaction;

2) determining a derivative of the growth curve, wherein the derivative is determined with respect to cycle number or time; and

3) calculating a cycle number or time value associated with a characteristic of the derivative;

b) deriving a calibration curve from the threshold values determined for the known starting quantities of the calibration nucleic acid sequence in the calibration samples; and

c) determining the starting quantity of the target nucleic acid sequence in the test sample using the calibration curve and the threshold value determined for the target sequence.

37. The apparatus of claim 36, wherein each of the threshold values comprises a cycle number.

38. The apparatus of claim 36, wherein each of the threshold values comprises an elapsed time of amplification.

39. The apparatus of claim 36, wherein the controller is programmed to perform steps (a2) and (a3) by determining the second derivative of the growth curve and calculating the cycle number or time value as the location, in cycles or in time of amplification, of a maximum of the second derivative.

40. The apparatus of claim 36, wherein the controller is programmed to perform steps (a2) and (a3) by determining the second derivative of the growth curve and calculating the cycle number or time value as the location, in cycles or in time of amplification, of a minimum of the second derivative.

41. The apparatus of claim 36, wherein the controller is programmed to perform steps (a2) and (a3) by determining the second derivative of the growth curve and calculating the cycle number or time value as the location, in cycles or in time of amplification, of a zero-crossing of the second derivative.

42. The apparatus of claim 36, wherein the controller is programmed to perform steps (a2) and (a3) by determining the first derivative of the growth curve and calculating the cycle number or time value as the

location, in cycles or in time of amplification, of a maximum of the first derivative.

43. The apparatus of claim 36, wherein the characteristic of the derivative comprises a maximum of the second derivative, and wherein the controller is programmed to perform steps (a2) and (a3) by:

calculating second derivative values of the growth curve, with respect to cycle number or time, at a number of different measurement points to yield a plurality of second derivative data points;

fitting a second curve to at least three of the second derivative data points; and

calculating the cycle number or time value as the location, in cycles or in time of amplification, of a positive peak of the second curve.

44. The apparatus of claim 43, wherein the cycle number or time value at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three second derivative data points.

45. The apparatus of claim 36, wherein the characteristic of the derivative comprises a minimum of the second derivative, and wherein the controller is programmed to perform steps (a2) and (a3) by:

calculating second derivative values of the growth curve, with respect to cycle number or time, at a number of different measurement points to yield a plurality of second derivative data points;

fitting a second curve to at least three of the second derivative data points; and

calculating the cycle number or time value as the location, in cycles or in time of amplification, of a negative peak of the second curve.

46. The apparatus of claim 45, wherein the cycle number or time value at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three second derivative data points.

47. The apparatus of claim 36, wherein the characteristic of the derivative comprises a maximum of the first derivative, and wherein the controller is programmed to perform steps (a2) and (a3) by:

calculating first derivative values of the growth curve, with respect to cycle number or time, at a number of different measurement points to yield a plurality of first derivative data points;

fitting a second curve to at least three of the first derivative data points; and

calculating the cycle number or time value as the location, in cycles or in time of amplification, of a peak of the second curve.

48. The apparatus of claim 47, wherein the cycle number or time value at the peak of the second curve is calculated using ratios of determinants, and wherein the determinants are calculated using the three first derivative data points.

49. The apparatus of claim 36, wherein the characteristic of the derivative comprises a zero-crossing of the second derivative, and wherein the controller is programmed to perform steps (a2) and (a3) by:

calculating second derivative values of the growth curve at a number of different measurement points to yield a plurality of second derivative data points; and

calculating the cycle number or time value at the zero-crossing by interpolation between at least two of the second derivative data points.